Journal of Surgical Oncology 1999;71:147–154

# Expression of p27Kip1 and Cyclin D1 Proteins Is Inversely Correlated and Is Associated With Poor Clinical Outcome in Human Gastric Cancer

SEHWAN HAN, MD,[1]* HONG-YONG KIM, MD,[1] KYEONGMEE PARK, MD,[2] MYUNG-SOO LEE, MD,[1] HONG-JOO KIM, MD,[1] AND YOUNG-DUCK KIM, MD[1]

[1]Department of Surgery, Inje University Sanggye Paik Hospital, Seoul, Korea
[2]Department of Pathology, Inje University Sanggye Paik Hospital, Seoul, Korea

**Background and Objectives:** p27Kip1 is an inhibitor of cyclin-dependent kinases and is speculated to be a potential prognostic indicator in numerous human cancers. We investigated expression of p27Kip1 along with cyclin D1 in gastric cancer to estimate the clinical utility of p27Kip1.
**Methods:** Immunohistochemical assay for p27Kip1 and cyclin D1 proteins was performed in 64 patients with primary gastric cancer. Correlation between p27Kip1 expression and clinical-biological parameters including patient survival was analyzed.
**Results:** p27Kip1 expression was suppressed in 40 (62.5%) of 64 gastric cancer patients and cyclin D1 was overexpressed in 22 (34.4%) out of 64. Expression of p27Kip1 was significantly reduced in poorly differentiated cancers (82.1%, 23/28; $P = 0.015$) and was also reduced in the tumors with high S-phase fraction (86.7%, 26/30) compared with tumors showing low S-phase fraction (41.2%, 14/34; $P = 0.0002$). Expression of p27Kip1 and cyclin D1 was inversely correlated ($P = 0.021$). In univariate analysis, extent of the disease ($P < 0.001$), expression of cyclin D1 ($P = 0.0001$), and reduced expression of p27Kip1 ($P = 0.0006$), were statistically significant to predict patient's outcome, but depth of invasion ($P = 0.008$) and pathologic stage ($P = 0.009$) emerged as significant prognostic indicators in multivariate analysis.
**Conclusion:** Expression of p27Kip1 is closely linked with cell proliferation and differentiation of human gastric cancer. p27Kip1 seems to have potential as a prognostic marker in the management of gastric cancer patients. *J. Surg. Oncol. 1999:71:147–154.* 

KEY WORDS: **cell proliferation; differentiation; immunohistochemistry; prognosis**

## INTRODUCTION

The cyclin-dependent kinase (cdk) inhibitor p27Kip1 is known to play a central role in negative regulation of cell growth. Overexpression of p27Kip1 is induced by transforming growth factor (TGF)-beta and results in G1 arrest [1]. Levels of p27Kip1 are high in the quiescent cells but decrease in the cycling cells when they get sequestered into excessive cyclin D-cdk complexes. Cyclin D1 is thought to be a primary cyclin in growing cells of human and is named G1 cyclin [2]. Events required to prepare the cell for entry into S-phase are mediated by the D- and E-type cyclins. The cell cycle control pathway governed by the D-type cyclins is the one most com-

*Correspondence to: Sehwan Han, MD, Department of Surgery, Inje University Sanggye Paik Hospital, 761-1 Sanggye-Dong, Nowon-Gu, 139-707 Seoul, Korea. Fax No.: 82 2 933-9389.
E-mail: shwhan@unitel.co.kr
Accepted 4 April 1999

monly mutated in human cancers. One of the well-established aspects of cyclin D1 overexpression in culture cells is a shortened G1 phase, resulting in a more rapid entry into S-phase and increased cell proliferation [3]. However, in those squamous cell carcinomas of the head and neck that are cyclin D1-negative in immunohistochemistry (IHC), proliferation may be driven by molecules other than those of the cyclin D family [4]. Therefore, cell cycle progression by cyclin D1 could be counteracted by various inhibitors of cdks.

Reduced expression of p27Kip1 has been reported in a number of human cancers, including breast, pituitary, colon, and gastric cancers [5–9]. Loss of cell growth control driven by p27Kip1 has been reported to be associated with aggressive biologic behavior of cancer cells in vivo. Mutations of p27Kip1 gene are rarely detected in human cancer at DNA level [10–13]. However, high levels of p27Kip1 mRNA were found in approximately 50% of primary breast cancer [14]. Reduced expression of p27Kip1 was also detected in 57% of stomach cancer patients, while no gross alteration of p27Kip1 gene was observed by Southern blot analysis [15]. Decrease of p27Kip1 positive cells significantly correlated with advanced stage, depth of tumor invasion, and lymph node metastasis in the report from Mori et al. [8]. Consequently, reduction of p27Kip1 expression may be an indicator of high-grade malignancy in stomach cancer.

Whether the changes in p27Kip1 protein seen in epithelial dysplasias and carcinomas are a primary event or are simply a consequence of increased cell proliferation has yet to be determined. There is evidence to suggest that abnormal p27Kip1 protein expression in cancer is predominantly mediated at the protein level, through changes in ubiquitin-proteasome-dependent degradation, without changes at the mRNA level [7]. Therefore, levels of p27Kip1 could be controlled by post-transcriptional mechanisms, and this implies that p27Kip1 expression is primarily regulated at the level of protein turnover [9]. p27Kip1 imposes an inhibitory threshold for cdk activation, and changes in p27Kip1 expression might readily occur through means other than mutation of p27Kip1 gene. Consequently, changes in p27Kip1 protein contribute to biologic behavior of cancers by unregulating the cell kinetics.

In the study reported here, we investigated the mechanism of cell cycle control of p27Kip1 and cyclin D1 in human gastric cancer by correlating two growth-regulating molecules with proliferative activity and biologic parameters of cancers. Additionally, clinical utility of p27Kip1 as a prognostic variable is also described.

## MATERIALS AND METHODS

Medical records and archival pathology tissues from 64 stomach cancer patients who underwent gastric resection at Inje University Sanggye Paik Hospital, Seoul, Korea between January 1994 and December 1995 were evaluated. Important selection criteria for entry to the study were feasible freshness of cancer tissues for flow cytometry analysis, and immunohistochemical assay of cyclin D1 and p27Kip1 protein. All patients underwent radical subtotal or total gastrectomy with more than D2 lymph node dissection. Mean age of studied patients was 61 years, ranging from 23 to 75 years: male patients' mean age was 41 (64.6%); female patients' mean age was 23 (35.4%). The stage of each patient was determined according to the TNM classification of the International Union Against Cancer; 26 with stage I (40.6%), nine with stage II (14.1%), 16 with stage III (25.0%), and 13 with stage IV (20.3%). Postoperative adjuvant chemotherapy, which was applied to all patients except those who had early gastric cancer (EGC), included 5-FU (600 mg/m$^2$), mitomycin (20 mg/m$^2$), and leucovorin (15 mg/m$^2$) every 3 weeks for six cycles.

**Cell cycle analysis.** Flow cytometry analysis was performed on cell suspensions from stomach cancers obtained by mechanical disaggregation of tumor materials. After centrifugation, supernatants were discarded, and the cell pellets were resuspended in 250 μl of buffer solution (10 mM Citrate, pH 7.5, 20 mM NaCl, 20 mM $MgCl_2$). After adding 10 μl/ml of trypsin, trypsin inhibitor, and DNase-free RNase, nuclei were incubated at room temperature for 30 min. DNA staining was obtained with 500 μl of propidium iodide solution (PI; Molecular Probes, Eugine, OR) in PBS (100 μl/ml PI, 0.1% Triton X-100, 1% FCS) for 1 hr at 4°C in the dark, followed by flow analysis. The DNA fluorescence was analyzed using a FACScan (Becton-Dickinson, Bedford, MA). Data acquisition was performed using the Cell Fit software (Becton-Dickinson) and data analysis using the Phoenix Flow System Multicycle AV software.

The results were expressed as the frequency distribution of DNA cell content; normal DNA histograms were characterized by a peak corresponding to the DNA content of G0/G1 diploid cells. Clonal DNA abnormality (aneuploidy) was identified by the presence of an accessory peak generally shifted to the right of the G0/G1 diploid peak. The percentage of aneuploid cells was defined as the percentage of cells in the G0/G1 aneuploid peak with respect to those in the G0/G1 diploid peak. Diploid tumors were considered as those with 0% aneuploid cells.

**Immunohistochemical assay.** The same paraffin blocks as for flow cytometry from gastric cancer patients were retrieved and neoplastic tissues of these gastric cancers were examined for expression of cyclin D1 and p27Kip1 protein using the avidin-biotin complex (ABC) immunoperoxidase method. We used commercially available monoclonal antibody; NCL-CYCLIN D1-GM (1:50 dilution) for cyclin D1 protein assay (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), mouse

anti-p27Kip1 monoclonal antibody: G173-524 (1:450 dilution) for p27Kip1 protein assay (Pharmingen, San Diego, CA). Immunostaining was performed as described previously [16]. Counterstaining with hematoxylin was done after ABC immunostaining and two pathologists evaluated immunohistochemical staining separately without information of patients' outcome data. Two pathologists reviewed the slides if interpretation of the immunohistochemical analysis was different. Three separate blocks containing malignant cells were stained and scored by calculating the stained cells from 500 observed cancer cells in percentage. Sections of stomach cancer observed to express homogenous and/or intense nuclear immunohistochemical staining for the p27Kip1 and cyclin D1 protein in more than 5% of the observed field were considered to be positive for expression (Fig. 1A,B).

Data for the immunohistochemical assay were merged with clinicopathologic characteristics of patients to determine the frequency of p27Kip1 and cyclin D1 proteins expression, and to evaluate the cyclin D1 and p27Kip1 for their implication as cell cycle regulators in human gastric cancer by correlating expression of two molecules with proliferative activity of the cancer cells. Statistical analyses were performed using the Statistical Package for the Social Science program 7.5 version (SPSS Inc., Chicago, IL). Correlation between clinical parameters and expression of cyclin D1 and p27Kip1 protein was estimated by the Mantel-Haenszel two-tailed chi-square test. Survival analysis was carried out by using a log-rank test, and we used the Cox proportional hazard model for multivariate analysis of studied prognostic variables.

## RESULTS

Expression of p27Kip1 protein was markedly suppressed in 40 patients (62.5%) out of 64. When we analyzed the correlation of p27Kip1 expression with clinicopathologic parameters of the patients, expression of p27Kip1 correlated with neither pathologic stage of the disease ($P = 0.945$) nor depth of tumor infiltration ($P = 0.600$). However, expression of p27Kip1 was significantly reduced in poorly differentiated cancers (82.1%, 23/28; $P = 0.015$) and was also reduced in the tumors with high S-phase fraction (86.7%, 26/30) compared with the tumors showing low S-phase fraction (41.2%, 14/34; $P = 0.0002$). This finding was also observed when we analyzed expression of p27Kip1 according to Lauren's classification of the tumors. According to Lauren's classification, 37 patients (57.8%) had intestinal-type tumor and 27 (42.2%) had diffuse-type tumor. Expression of p27Kip1 significantly decreased in diffuse-type cancers (Table I). Interestingly, expression of p27Kip1 was limited to the nonproliferating cells in the peripheral area of lymphoid follicles (Fig. 1C), while its expression was absent in actively proliferating cells of the germinal center.

We also performed immunohistochemical assay for cyclin D1 protein with the same tumor tissues. Expression of cyclin D1 protein was observed in 22 patients (34.4%) of a total 64 patients. Thirty-eight patients (59.4%) had diploid tumor, while 26 patients (40.6%) had aneuploid tumor. Cyclin D1 expression increased in aneuploid cancers but the result had no statistical significance ($P = 0.431$). Mean value of S-phase fraction measured by flow cytometry analysis was 15.4%. Increased expression of cyclin D1 was observed more frequently in the tumors with high levels of S-phase fraction, but the difference had only borderline significance ($P = 0.052$). Cyclin D1 expression slightly increased in diffuse-type tumors, but there was no significant correlation between cyclin D1 expression and Lauren's classification ($P = 0.492$). Furthermore, high levels of cyclin D1 expression were observed in a significant proportion (50.0%) of well-differentiated gastric cancers. When we estimated the frequency of cyclin D1 expression according to the depth of tumor infiltration, there was no significant correlation between cyclin D1 expression and depth of tumor infiltration ($P = 0.705$). Moreover, cancer cells of EGC showed an increased expression of cyclin D1 in 34.6% (9 of 26), and the result demonstrated that expression of cyclin D1 was present even in the early stage of the disease. This finding was demonstrated similarly when we analyzed expression of cyclin D1 according to the extent of the disease using TNM classification—which showed persistent expression of cyclin D1 through progress of the disease. However, we could not observe any correlation between lymph node metastases and cyclin D1 expression ($P = 0.407$).

Correlation between the expression of cyclin D1 and p27Kip1 in the same cancer specimens from the individual patient was evaluated. Of 22 tumors with cyclin D1 overexpression, p27Kip1 expression was absent in 18 tumors (81.8%). Only four tumors (18.2%), in which cyclin D1 was overexpressed, showed persistent expression of p27Kip1. Figure 2 shows the inverse correlation between expression of p27Kip1 and cyclin D1 proteins ($P = 0.021$).

In survival analysis, there was significant correlation between clinical outcome of the patients and two molecular markers in univariate analysis. Of 26 patients (40.6%) who had recurrent disease, 16 patients (61.5%) exhibited increased expression of cyclin D1, while increased expression of cyclin D1 was demonstrated in only six patients (15.8%) who were free of the disease at the end of the study period (Fig. 3). The opposite finding was observed when we analyzed treatment outcome according to expression of p27Kip1. Expression of p27Kip1 was reduced in the 23 (88.5%) out of 26 patients with recurrent disease (Fig. 4). However, patho-

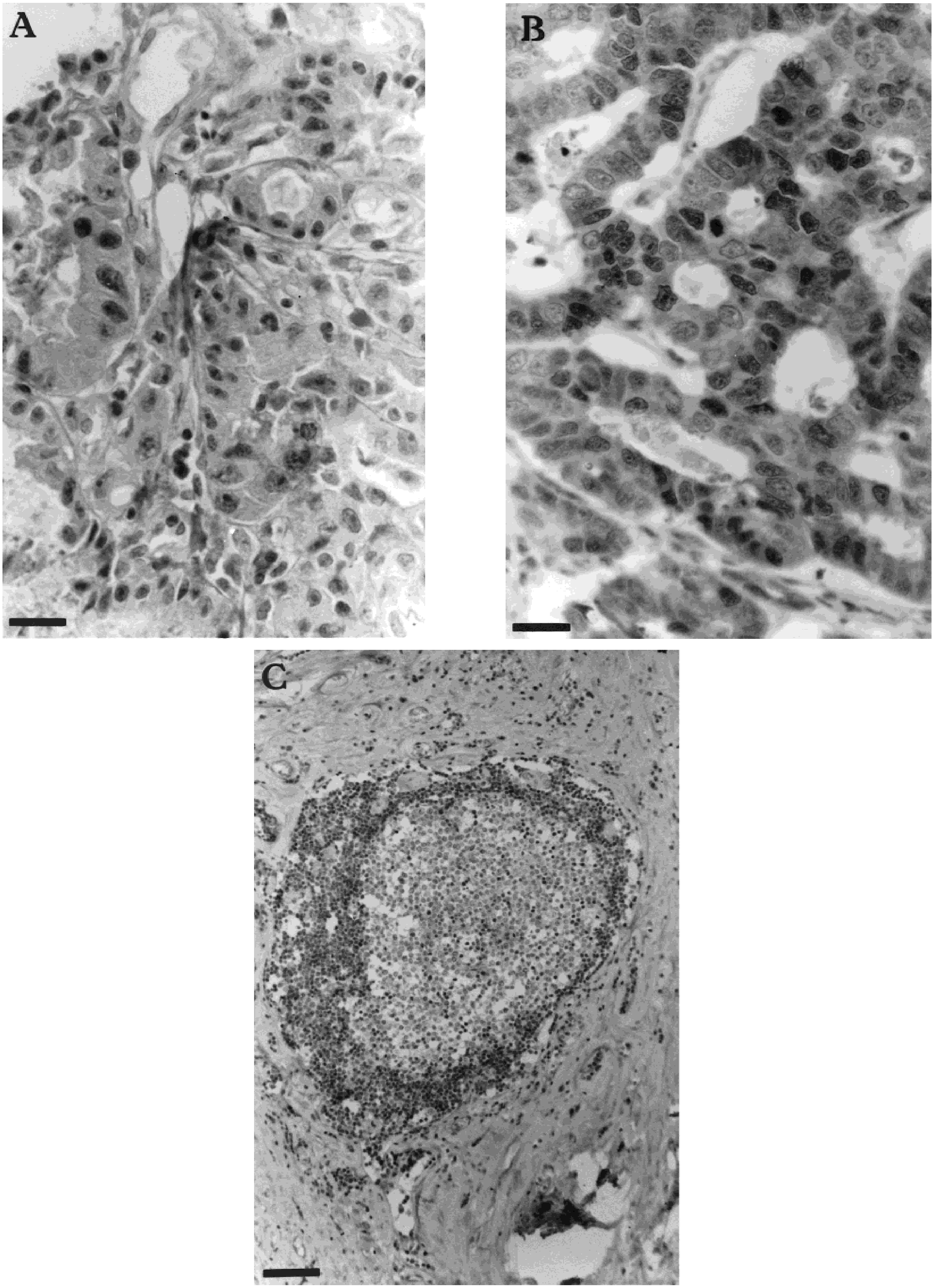


Fig. 1. Immunohistochemical staining of cyclin D1 and p27Kip1 protein. **(A)** Nuclear staining of cyclin D1 in gastric cancer cells. Scale bar $= 25$ μm (×400). **(B)** Nuclei of cancer cells are stained with p27Kip1. Scale bar $= 25$ μm (×400). **(C)** p27Kip1 immunostaining is limited to the peripheral, nonproliferating area of lymphoid follicle. Scale bar $= 50$ μm (×200).

**TABLE I. Correlation Between Clinicopathologic Data of Gastric Cancer Patients and Expression of Cyclin D1 and p27Kip1 Protein**

| | Cyclin D1 expression (%) | | | p27 expression (%) | | |
|---|---|---|---|---|---|---|
| Variables | Low | High | *P*-value | Low | High | *P*-value |
| Depth of invasion | | | NS[a] | | | NS |
| T1 | 17/26 (65.4) | 9/26 (34.6) | | 16/26 (61.5) | 10/26 (38.5) | |
| T2 | 5/11 (45.5) | 6/11 (54.5) | | 5/11 (45.5) | 6/11 (54.5) | |
| T3 | 12/13 (92.3) | 1/13 (7.7) | | 10/13 (76.9) | 3/13 (23.1) | |
| T4 | 8/14 (57.1) | 6/14 (42.9) | | 9/14 (64.3) | 5/14 (35.7) | |
| L/N metastasis | | | NS | | | NS |
| Negative | 16/26 (61.5) | 10/26 (38.5) | | 18/26 (69.2) | 8/26 (30.8) | |
| Positive | 26/38 (68.4) | 12/38 (31.6) | | 22/38 (57.9) | 16/38 (42.1) | |
| Stage | | | NS | | | NS |
| I | 15/26 (57.7) | 11/26 (42.3) | | 16/26 (61.5) | 10/26 (38.5) | |
| II | 7/9 (22.2) | 2/9 (77.8) | | 6/9 (66.7) | 3/9 (33.3) | |
| III | 12/16 (75.0) | 4/16 (25.0) | | 10/16 (62.5) | 6/16 (37.5) | |
| IV | 8/13 (61.5) | 5/13 (38.5) | | 8/13 (61.5) | 5/13 (38.5) | |
| Lauren's classification | | | NS | | | 0.004 |
| Intestinal | 25/37 (67.6) | 12/37 (32.4) | | 17/37 (45.9) | 20/37 (54.1) | |
| Diffuse | 17/27 (62.9) | 10/27 (37.1) | | 23/27 (85.2) | 4/27 (14.8) | |
| Differentiation | | | NS | | | 0.015 |
| Well | 2/4 (50.0) | 2/4 (50.0) | | 1/4 (25.0) | 3/4 (75.0) | |
| Moderate | 19/24 (79.2) | 5/24 (20.8) | | 10/24 (41.7) | 14/24 (58.3) | |
| Poor | 16/28 (57.1) | 12/28 (42.9) | | 23/28 (82.1) | 5/28 (17.9) | |
| Signet ring | 5/8 (62.5) | 3/8 (37.5) | | 6/8 (75.0) | 2/8 (25.0) | |
| Ploidy | | | NS | | | NS |
| Diploid | 28/38 (73.7) | 10/38 (26.3) | | 25/38 (65.8) | 13/38 (34.2) | |
| Aneuploid | 14/26 (53.8) | 12/26 (46.2) | | 15/26 (57.7) | 11/26 (42.3) | |
| S-phase | | | 0.052 | | | 0.0002 |
| <15% | 26/34 (76.5) | 8/34 (23.5) | | 14/34 (41.2) | 20/34 (58.8) | |
| ≥15% | 16/30 (53.3) | 14/30 (46.7) | | 26/30 (86.7) | 4/30 (13.3) | |

[a]NS, statistically not significant.

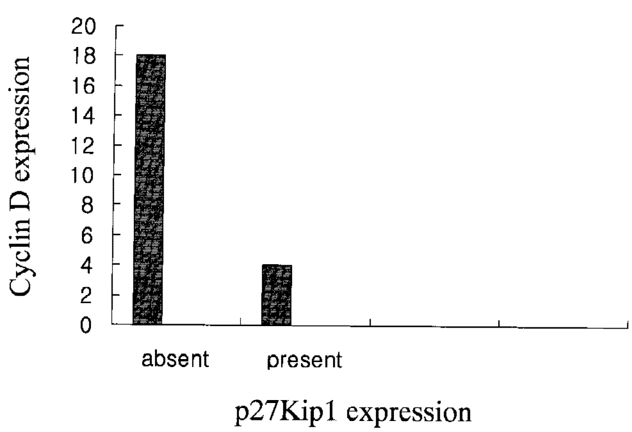


Fig. 2. Cyclin D1 expression according to the expression status of p27Kip1 protein. Expression rate of cyclin D1 is significantly decreased in the tumors in which p27Kip1 expression are preserved ($P = 0.021$).

logic stage ($P = 0.009$) and depth of invasion ($P = 0.008$) were the significant prognostic factors in multivariate analysis with median follow-up period of 37 months, ranging from 19 months to 51 months (Table II). Results of survival analyses are summarized in Table II.

## DISCUSSION

In the results of present study, p27Kip1 expression was significantly reduced in the tumors with high S-phase fraction ($P = 0.002$) and there was inverse correlation between cyclin D1 and p27Kip1 expression ($P = 0.021$). Therefore, p27Kip1 may function as a cell cycle regulator by downregulating cyclin D1 expression in the growth of human gastric cancer. Induction of cyclin D1 is an early response to mitogenic stimulation in several cell types, and cyclin D1 is a key regulator for the cell cycle progression through the G1 phase [17]. The primary target of cyclin D1 is the checkpoint in the late G1 phase, known as the “restriction point.” There are some conflicting results of the effects of cyclin D1 upon cell kinetics. A study from Japan reported that cyclin D1 gene is rarely amplified in human gastric cancer tissues or cell lines [18], but we demonstrated that a significant proportion (34.4%) of gastric cancers expressed cyclin D1 protein. Results of the present study may be indirect evidence for the existence of cyclin D1-dependent pathway in the cell proliferation of human gastric cancer, since cyclin D1 expression inversely correlated with p27Kip1 expression ($P = 0.021$), which was significantly associ-

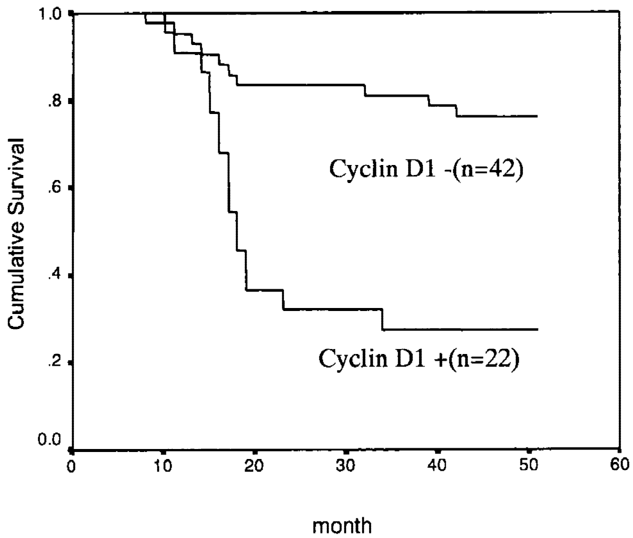


Fig. 3. Cumulative survival of the patients with gastric cancer according to cyclin D1 expression status. Patients with tumors expressing cyclin D1 had poorer survival than other patients ($P = 0.0001$ by log-rank test).

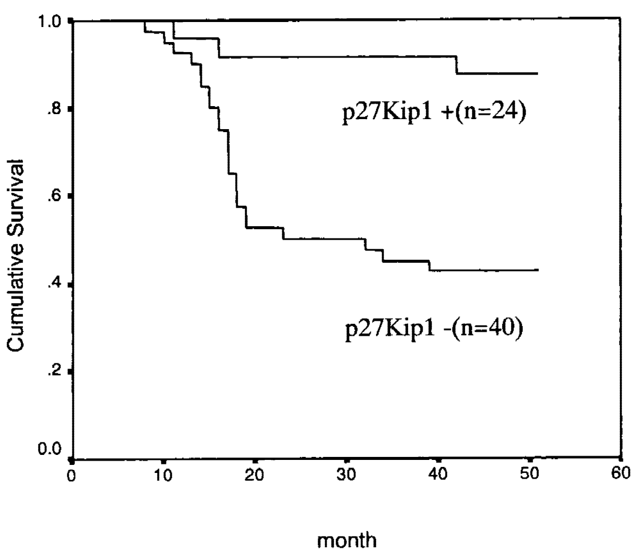


Fig. 4. Cumulative survival of the patients according to the expression of p27Kip1 protein. Patients with tumors lack of p27Kip1 expression had poorer survival than other patients ($P = 0.0006$ by log-rank test).

ated with proliferative activity of gastric cancer. However, there was no significant correlation between cyclin D1 expression and S-phase in the results of present study ($P = 0.659$). In human breast cancer cells, it has been reported that cyclin D1 induction shortens G1 phase [19]. Wang et al. [20] also reported that overexpression of cyclin D1 deregulates cell proliferation in transgenic mice. More than 1/3 of human breast cancers may contain excessive levels of cyclin D1 protein [21], but increased gene dosage of cyclin D1 does not seem to be the only mechanism that resulted in increased protein expression as detected by immunostaining [22]. Frequency of cyclin D1 protein abnormalities is considerably higher than DNA amplification, and the level of cyclin D1 in human gastric cancer might be regulated by post-transcriptional mechanisms as in breast cancer. Other unknown factors, such as cyclin E and cdk inhibitors other than p27Kip1, can also influence the cell proliferation in human gastric cancer, because genetic changes of p27Kip1 and cyclin D1 at the DNA levels have not yet been detected in human gastric cancer. Moreover, a considerable number of gastric cancers that expressed neither cyclin D1 nor p27Kip1 were present in the results of this study.

In human breast cancer, high levels of cyclin D1 protein are seen in well-differentiated estrogen receptor (ER) -positive tumors [23,24]. However, we could not find any relationship between cyclin D1 expression and cell differentiation in gastric cancers ($P = 0.492$). Expression of cyclin D1 increased in a certain proportion of differentiated cancers, but undifferentiated cancers also expressed cyclin D1 in the present study. Increased expression of cyclin D1 can inhibit cell growth, induce differentiation, and enhance apoptosis in some mammary epithelial cells. These effects might be due, at least in part, to the fact that these derivatives displayed increased expression of the p27Kip1 inhibitory proteins [25]. Fredersdorf et al. observed the correlation between the high level of p27Kip1 expression and cyclin D1 in human breast and colon cancer cells [26]. They postulated that overexpression of cyclin D1 can induce the expression of p27Kip1, either directly or indirectly, via the feedback regulatory loop. The present study demonstrates a significant association between p27Kip1 expression and differentiation of the cancer cells ($P = 0.015$). In the noncancerous lymphatic tissues adjacent to cancerous lesions, expression of p27Kip1 was limited to the nonproliferating cells in the peripheral area of lymphoid follicles (Fig. 1C), while its expression was absent in the actively proliferating cells of the germinal center. p27Kip1 seems to have a differentiating signal in human gastric tissues. However, in the present study, we observe that cyclin D1 expression is inversely correlated with the expression of p27Kip1 in gastric cancer ($P = 0.021$). This result implicates that there exists a feedback inhibitory loop between cyclin D1 and p27Kip1 in human gastric cancers. This reciprocal effect was also displayed in colorectal cancer [27] and could be explained by sequestration of p27Kip1 protein with excessive production of cyclin D1 in growing cells. Expression of cyclin D1 may vary with tissue types or cell clones and may be under the different regulatory pathway according to the organ tissues.

Reduced expression of p27Kip1 has been known to be

**TABLE II. Survival Analysis According to the Prognostic Variables of Gastric Cancer**

| Variables | Univariate analysis[a] | Multivariate analysis[b] | RR(CI)[c] |
|---|---|---|---|
| Differentiation | 0.108 | 0.053 | 0.6 (0.3–1.0) |
| Ploidy | 0.598 | 0.561 | 1.2 (0.6–2.2) |
| S-phase | 0.477 | 0.726 | 0.8 (0.4–1.6) |
| Depth of invasion | <0.001 | 0.008 | 2.0 (1.2–3.4) |
| Lymph node metastasis | 0.019 | 0.535 | 1.2 (0.6–2.3) |
| Stage | <0.001 | 0.009 | 1.8 (1.1–2.9) |
| Cyclin D1 | <0.001 | 0.947 | 0.9 (0.4–1.9) |
| p26 | <0.001 | 0.588 | 0.8 (0.4–1.5) |

[a]Univariate analysis was conducted using log-rank test; results are shown as *P*-value.
[b]Multivariate analysis was conducted using Cox Regression Model; results are shown as *P*-value.
[c]Relative risk determined by Cox Regression Model; 95% confidence intervals in parentheses.
*P*-value less than 0.05 was considered as statistically significant.

an indicator of high-grade malignancy and to be associated with poor survival of patients in numerous human cancers [5–9,28–30]. However, there was no significant correlation between p27Kip1 expression and stage of the disease ($P = 0.945$) and depth of tumor infiltration ($P = 0.600$) in the present study, whereas other studies showed significant correlation between p27Kip1 expression and aggressiveness of the tumors [5–9]. Multivariate analysis revealed that stage of the disease ($P = 0.009$) and depth of invasion ($P = 0.008$) were independent prognostic factors to predict the outcome of the patients with gastric cancer. When we analyzed the patients' outcomes excluding stage IV populations, cyclin D1 expression ($P = 0.0001$) and reduced expression of p27Kip1 ($P = 0.0006$) emerged as significant prognostic factors. The finding of present study may be indirect evidence of an aggressive biologic property of the tumors that lack expression of p27Kip1. However, we are not able to conclude that cyclin D1 and p27Kip1 are useful prognostic indicators at this point, because the study was conducted on small number of patients and treatment outcome was estimated in a relatively short period of follow-up. Additional study with larger patient population seems necessary to elucidate the clinical utility of cyclin D1 and p27Kip1 as useful prognostic factors in human gastric cancer.

## CONCLUSIONS

Cyclin D1 expression seems to be downregulated by p27Kip1 in certain clones of human gastric cancer. However, it is obviously clear that a single alteration of one gene does not result in carcinogenesis and there may exist other clones in human gastric cancer that are under regulation of other than cyclin D1 or p27Kip1. Consequently, clinical utility of cyclin D1 and p27Kip1 as prognostic indicators may be limited in a certain proportion of patients with gastric cancer. Altered expression of cyclin D1 and p27Kip1 may represent an aggressive phenotype of human gastric cancer with increased proliferative activity.

## ACKNOWLEDGMENTS

The authors thank Hee-Jeong Kim for technical assistance in flow cytometry analysis and Sun-Hee Song for statistical analyses.

## REFERENCES

1. Slingerland JM, Hengst L, Pan CH, et al.: A novel inhibitor of cyclin-Cdk activity detected in transforming growth factor β-arrested epithelial cells. Mol Cell Biol 1994;14:3683–3694.
2. Sherr CJ: Mammalian G1 cyclins. Cell 1993;73:1059–1165.
3. Mueller A, Odze R, Jenkins TD, et al.: A transgenic mouse model with cyclin D1 overexpression results in cell cycle, epidermal growth factor receptor, and p53 abnormalities. Cancer Res 1997; 57:5542–5549.
4. Kotelnikov VM, Coon IV JS, Mundle S, et al.: Cyclin D1 expression in squamous cell carcinomas of the head and neck and oral mucosa in relation to proliferation and apoptosis. Clin Cancer Res 1997;3:95–101.
5. Catzavelos GC, Bhattacharia N, Ung YC, et al.: Decreased levels of the cell-cycle inhibitor p27Kip1protein: prognostic implications in primary breast cancer. Nature Med 1997;3:227–230.
6. Lloyd RV, Jin L, Qian X, Kulig E: Aberrant p27kip1 expression in endocrine and other tumors. Am J Pathol 1997;150:401–407.
7. Loda M, Cukor B, Tam SW, et al.: Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nature Med 1997;3:231–234.
8. Mori M, Mimori K, Shiraishi T, et al.: p27 expression and gastric carcinoma. Nature Med 1997;3:593.
9. Porter PL, Malone KE, Heagerty PJ, et al.: Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nature Med 1997;3:222–225.
10. Spirin KS, Simpson JF, Takeuchi S, et al.: p27/Kip1 mutation found in breast cancer. Cancer Res 1996;56:2400–2404.
11. Ferrando AA, Balbin M, Pendas AM, et al.: Mutational analysis of the human cyclin-dependent kinase inhibitor p27kip1 in primary breast carcinomas. Hum Genet 1996;97:91–94.
12. Kawamata N, Morosetti R, Miller CW, et al.: Molecular analysis of the cyclin-dependent kinase inhibitor gene p27/Kip1 in human malignancies. Cancer Res 1995;55:2266–2269.
13. Ponce-Castaneda MV, Lee MH, Latres E, et al.: p27Kip1:chromosomal mapping to 12p12–12p13.1 and absence of mutations in human tumors. Cancer Res 1995;55:1211–1214.
14. Rasmussen UB, Wolf C, Mattei MG, et al.: Identification of a new interferon-alpha-inducible gene (p27) on human chromosome 14q32 and its expression in breast carcinoma. Cancer Res 1993; 53:4096–4101.
15. Yasui W, Kubo Y, Semba S, et al.: Reduced expression of cyclin-dependent kinase inhibitor p27Kip1 is associated with advanced

stage and invasiveness of gastric carcinomas. Japanese J Cancer Res 1997;88:625–629.
16. Han S, Yun IJ, Noh DY, et al.: Abnormal expression of four novel molecular markers represents a highly aggressive phenotype in breast cancer. Immunohistochemical assay of p53, nm23, erbB-2, and cathepsin D protein. J Surg Oncol 1997;65:22–27.
17. Strauss M, Lukas J, Bartek J: Unrestricted cell cycle and cancer. Nature Med 1995;1:1245–1246.
18. Akama Y, Yasui W, Yokozaki H, et al.: Frequent amplification of the cyclin E gene in human gastric carcinomas. Japanese J Cancer Res 1995;86:617–621.
19. Musgrove EA, Lee CS, Buckley MF, Sutherland RL: Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle. Proc Natl Acad Sci USA 1994;91:8022–8026.
20. Wang TC, Cardiff RD, Zukerberg L, et al.: Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature 1994;369:669–671.
21. Bartkova J, Lukas J, Muller H, et al.: Cyclin D1 protein expression and function in human breast cancer. Int J Cancer 1994;57:353–361.
22. Zhang SY, Caamano J, Cooper F, et al.: Immunohistochemistry of cyclin D1 in human breast cancer. Am J Clin Pathol 1994;102:695–698.
23. Barnes DM: Cyclin D1 in mammary carcinoma. J Pathol 1997;181:267–269.
24. van Diest PJ, Michalides RJ, Jannink L, et al.: Cyclin D1 expression in invasive breast cancer. Correlation and prognostic value. Am J Pathol 1997;150:705–711.
25. Han EK, Begemann M, Sgambato A, et al.: Increased expression of cyclin D1 in a murine mammary epithelial cell line induces p27Kip1, inhibits growth, and enhances apoptosis. Cell Growth Differentiation 1996;7:699–710.
26. Fredersdorf S, Burns J, Milne AM, et al.: High level expression of p27 (kip1) and cyclin D1 in some human breast cancer cells: inverse correlation between the expression of p27 (kip1) and degree of malignancy in human breast and colorectal cancers. Proc Natl Acad Sci USA 1997;94:6380–6385.
27. Ciaparrone M, Yamamoto H, Yao Y, et al.: Localization and expression of p27Kip1 in multistage colorectal carcinogenesis. Cancer Res 1998;58:114–122.
28. Tsihlias J, Kapusta LR, DeBoer G, et al.: Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. Cancer Res 1998;58:542–548.
29. Yatabe Y, Masuda A, Koshikawa T, et al.: p27Kip1 in human lung cancers: Differential changes in small cell and non-small cell carcinomas. Cancer Res 1998;58:1042–1047.
30. Singh SP, Lipman J, Goldman H, et al.: Loss or altered subcellular localization of p27 in Barrett's associated adenocarcinoma. Cancer Res 1998;58:1730–1735.